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AUTHOR(S):

ODA, YOSHIFUMI; HANDA, HAJIME; KIELER, JØRGEN

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Induction and Transplantability of Rat Neurogenic Tumors*

Yoshifumi Oda, Hajime Handa

Department of Neurosurgery, Kyoto University Medical School

Jørgen Kieler

The Fibiger Laboratory,**

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Summary

96 rat neurogenic tumors were induced by transplacental administration of ethylnitrosourea (ENU) in late foetal life.

In most cases histological diagnosis was either malignant neurinoma (41%) or malignant oligodendroglioma (25%).

Dimethylbenzanthracene administrated in the seme way as ENU failed to induce the neurogenic tumors.

Seven ENU-induced neurogenic tumors were maintaned in serial transplantation. In the first transplantation generation some tumors having the same histological pattern showed marked differences in the growth speed. This might depend on the strength of the expression of the antigenicity of each tumor. After the second generation, each histologic type tended to exhibit the same latency and lifespan.

Introduction

Since the report of Druckley[5], many types of tumor have been induced by different nitroso-derivatives in various strains of animals[16)21)]. Although each derivatives has different optimal conditions for induction of tumors, all are organotropic. Irrespective of the route of administration, these chemicals mainly induce nervous system tumors, and are most effective in rat.

For comparative study of tumors in the human nevous syrstem, the transplacental administration of ENU provides an interesting approach. Almost all rats given certain amounts of ENU in the late embryonal period develop nervous system tumors in adult life.

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Key words : Carcinogen, Animal experiment, Glioma, Nervous tumor, Trasplantation, Ethylnitroso-urea.

**Ndr. Frihavnsgade 70, 2100, Copenhagen ϕ, Denmark, sponsored by the Danish Cancer Society

Many investigations have attempted to clarify the oncogenic properties and neurotropical nature of nitroso-derivatives, because their tumorigenic patterns and histological appearances are quite similar to human intracranial gliomas[14]

Materials and Methods

Rats : Inbred Wistar/AfHanMolFib. (Wistar/Fib.) rats of two to three months old were obtained from our animals colony maintained under 'minimal disease' condition at the Fibiger Laboratory.

ENU was obtained from Nakarai chemicals (Kyoto, Japan).

Induction of nervous tumors : 60 mg/kg of ENU was injected intraperitoneally in pregnant rats in the third stage of gestation. ENU was first dissolved in ethanol and then with 10 volumes of phosphate buffer solution (PBS). In some rats dimethylbenzanthracene (DMBA) (15mg/kg) was injected in the same way as ENU in order to reexamine NAPALKOV's report[13].

The offsprings were observed until spontaneous death or until the preterminal stage. The postmortem examination included inspection of the cranial cavity and spinal canal and removal of the brain and spinal cord. All tumors were examined histologically. This study concerns 110 rats which died or were killed in the preterminal stage. Rats aged less than three months at death were not included. No tumors were demonstrated in these animals.

Transplantation of tumors : ENU-induced neurogenic tumors were transplanted by subcutaneous inoculation into newborn syngeneic Wistar rats. Serial transplantations were carried out in 2-3 month old recipients of both sexes. The tumors to be transplanted were cut into small pieces with scissors and suspended in PBS. In quantitative studies single cell suspensions were prepared by mild homogenization of the tumor pieces in a Daunce glass homogenizer and filtration through a monolayer nylon mesh (pore size ; 80μ). Tumor cells were then counted with hemocytometer.

A spontaneous mammary tumor which originated in a non-treated Wistar rat was also transplanted in the same way.

Explantation and cultivation of tumors : Small (1-2mm) pieces of tumor tissues were placed at the bottom of T-25 or T-75 plastic Falcon flasks and preincubated for 1-2 hr at 37°C under 5% CO_2. When a liquid medium was added carefully to the cultures after preincubation, most of the explants remained fixed to the bottom. The medium (Fib 41B) was a modification of Eagle's medium (MEM), with a two-fold concentration of aminoacids, a four-fold concentration of vitamines and glutamine, and a 35% higher glucose concentration. This medium was fortified with 15% foetal calf serum (FCS). The cultures were incubated in a humid 5% CO_2 incubator for varying periods of time. For subcultivation, the cells were brought into suspension with the aid of trypsin. The medium was renewed 2-3 times weekly.

Results

Tumor induction : Thirty pregnant rats were divided into ten groups, of which nine received a single i.p. injection of ENU and one received DMBA. These rats produced 183 offspring (83M, 100F), of which 160 survived for more than 3 months. These were observed for 115-509 days.

In the ENU groups, 110 rats (50M, 60F) have so far died or been killed in the preterminal stage.

In 99 rats (47M, 52F), a total of 110 tumors were found. The location of these tumors and their occurrence in male and female rats are summarized in Table I. In most cases the histological diagnosis was either malignant neurinoma (41%) or malignant oligodendroglioma (25%), but mixed gliomas, astrocytomas, ependymomas and meningiomas were also seen.

Table. I. Tumor Induction by Ethylnitrosourea

group	nervous syst.	other org.	unknown	total no. of deaths	total no. in group
1	17	1	1	19	27
2	15	0	1	16	20
3	16	0	2	18	19
4	15	1	2	18	24
5	7	0	1	8	14
6	14	1	1	16	17
7	4	0	2	6	11
9	8	0	0	8	15
10	0	0	1	1	13
total	96	3	11	110	160
(*DMBA	0	1	1	2	3)

* DMBA : The group injected with dimethylbenzanthracene

Table. II. Classification and Latency Period of ENU-induced Tumor

Sex	central nervous system		peripheral nervous system		meningeal tumor	other tumor	total
	brain tumor	spinal intramed.	cranial nerve t.	spinal & periph. n. t.			
male (latency, days)	27 cases* (237±66)	2 cases (164±36)	14 cases (239±51)	7 cases (268±118)	0	1 case (241)	51
female (latency, days)	32 (260±71)	4 (208±102)	15 (277±102)	4 (253±46)	2 (182±39)	2 (296±113)	59
total (latency, days)	59 cases (250±69)	6 cases (193±84)	29 cases (259±82)	11 cases (262±95)	2 cases	3 cases	110 cases
	65 cases (244±72)		40 cases (260±85)				

* Cases refers to the number of tumors not of rats.
In 99 (47M 52F) rats a total of 110 (51M, 59F) tumor was found.

DMBA failed to produce the neurogenic tumors, but one abdominal adenocarcinoma was produced.

Classification and latency period are shown in Table II. There was no sex predominancy, and no differences in latency period between central (CNS) and peripheral nervous system (PNS) tumors.

Tumor Transplantation and Explantation :

A total of 12 ENU-induced neurogenic tumors and one spontaneous mammary carcinoma were transplanted into newborn rats. The results are shown in Table III For most tumors approximately $1x10^7$ cells were injected subcutaneously. For tumor T6, each received only $8.5x10^4$ cells, which may explain the negative result. The rats injected with T12 died

Table. Ⅲ. Summary of Transplanted Tumors

tumor no.	histology	1st generation			2nd generation			life-span (days)
		take	%	latency	take	%	letency	
T 1	perph. nerve neuri noma	10/10	100%	36± 5 days	11/11	100%	32±26days	47± 3 days
T 2	trigeminal n. neurinoma	3/3	100	34± 7	8/8	100	11± 4	17±11
T 3	trigeminal n. neurinoma	4/4	100	27± 0	19/19	100	12± 3	30± 6
T 4	mixed glioma	7/7	100	34±40	6/6	100	13± 4	over 100 days
T 5	oligodendrogl.	6/6	100	14± 9	5/5	100	19± 4	
T 6	oligodendrogl.	0/5	0					
T 7	astrocytoma	3/7	43	89±44	3/3	100	17± 0	
T 8	oligodendrogl.	5/9	56	45±40	4/4	100	13± 0	
(T 9	spont. mammary cancer	7/7	100	10± 0	7/7	100	13± 8)	
T10	ependymoma	4/4	100	27± 0				
T11	trigeminal n. neurinoma	3/3	100	27± 0				
T12	malignant oligodendrogl.	0/5	0	dead				
T13	brain tumor	0/4	under	observation				

Fig. 1. T1 tumor transplanted in vivo

Fig. 2. Section of T1 tumor

of unknown causes. The remaining 9 neurogenic tumors were transplantable in newborn animals (Fig. 1, 2), and seven were transplantable in adult recipients after the second transplant generation. Mammary carcinoma was also easily maintained in serial transplantation.

The latency periods in the first generation of transplantation varied considerably with the histological diagnosis. Usually CNS tumors showed a longer latency period than PNS tumors. However, in the second generation, this period was markedly reduced, and each histologic type tended to keep the same latency and life span after the second generation.

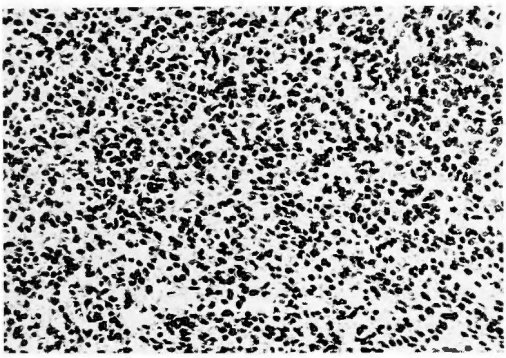

Fig. 3 T2 Tumor

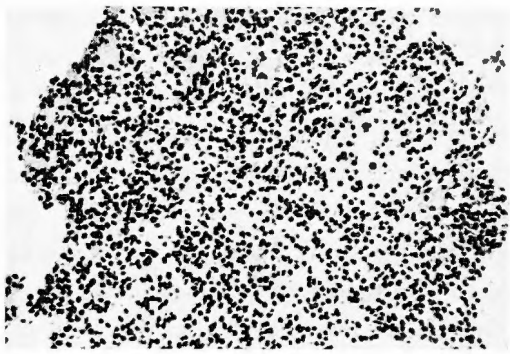

Fig. 4 T3 Tumor

Both T2 and T3 had the same histological pattern (Fig. 3, 4), though they differed greatly in growth speed. This might depend on the strength of the expression of the antigenicity of each tumor.

All of the transplantable neurogenic tumors were grown in tissue culture. On reinoculation into rats at a dose of $5x10^6$ cells or less, the cultured cells proved still to be tumorigenic. And these tumors closely retained the original histological appearances (Fig. 5).

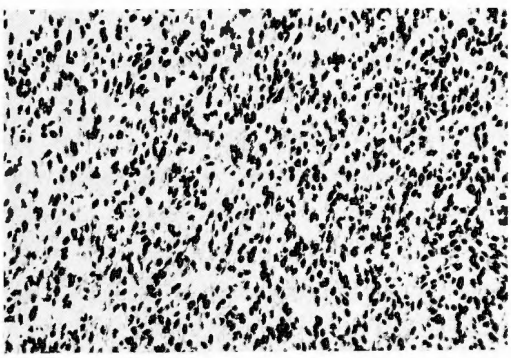

Fig. 5 T3 tumor reinoculated into syngeneic adult rats after explantation, showing the same histological appearance as the original T3 tumor

Discussion

After the first report on the carcinogenicity of nitroso-derivatives by MAGGEE et al. (1956)[12], DRUCKLEY[5] induced neuroginic rat tumors with methylnitrosourea (MNU) in 1964. Subsequently, many neurogenic tumors have been induced by nitroso-compounds in several species of animals, such as mouse, rabbit[16], dog[21] and hamster, but not in cat[3].

In 1969 WECHSLER et al.[22] reviewed the ENU-induced neurogenic rat tumors and commented on the possibility that exogeneous stimulation may play a role in the development

of human neurogenic tumors.

Rats are particularly susceptible to the tumorigenic action of ENU, and the transplacental route seems most effective. In Sprague-Dawley rats the overall spontaneous incidence of neuroginic tumors is only 0.44%[6], but such tumors are readily induced by a number of nitrosourea compound administrated by any of several routes[3)19)].

A large variety of tumors has been described. According to LANTOS[10], ENU-induced neurogenic tumors mainly originate from the subependymal glial plate. However, LAGEMAN and DIETS[9] also reported the development of tumors from perivascular adventitial cells.

In the present study neurinomas and oligodendrogliomas were the most common tumors induced by ENU. The differences in tumor induction period between the CNS and the PNS described by WECHSLER et al.[22] were not observed. In agreement with the studies of SCHREIBER et al.[15] on the influences of castration and teststeron adminstration on MNU oncogenicity, no sex prevalance of ENU-induced neurogenic tumors was observed in our study.

The action mechanisms of ENU have been examined biochemically. It has been assumed that both the carcinogenicity and the short term toxic effects of nitrosoamides result from the alkylation of macromolecules, in particular DNA. However, KNOX[8] has shown that cyanate ion is responsible for short-term effects and cytotoxicity. On the other hand, GOTH[7] reported that the excretion of 0-6-ethylguanine was quite slow in brain tissue, which might explain the neurotropic nature of ENU.

Like many other tumors, the neurogenic tumors exibit an increased metabolic rate and a high level of acid phosphatase[11]. Increased in cellularity and acid hydrolylase activity (N-acetyl-beta-glucosaminidase and beta-glucuronidase) as early as 20 days after prenatal ENU exposure were demonstrated in rat trigeminal nerve (SWENBERG et al.[20]). In spite of grossly normal appearance some of these nerves proved to be tumorigenic on inoculation into susceptible rats, and the increased enzyme activities were consequently interpreted as neoplastic rather than preneoplastic changes.

BIESSMANN[2] noted a difference in the composition of nonhistone proteins from neurogenic tumors and from normal tissues. This might be due to a difference in the chromation template activities.

Most of these tumors contain the nerve specific proteins S-100[1)18)23)] and 14-3-2[17]. However, according to DITTMANN et al.[4] S-100 is present in human glioblastomas only in approximately one-tenth of the amount in whole brain homogenate, whereas the glial acidic protein (GFA) was found to be enriched 2-4 times.

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References

1) Benda, P., Someda, K., Messer, J., Sweet, W. H., Morphological and immunochemical studies of rat glial and clonal strains propagated in culture, J. Neurosurg **34** : 310-323, 1971.

2) Biessmann, H., and Rajewsky, M. F., Nuclear protein patterns in developing and adult brain and in ethhylnitrosourea-induced neuroectodermal tumors of the rat. J Neurochem **24** 387-393, 1975.
3) Cravioto, H. M., Weiss, J. S., Geobel, H., H. Weiss, E. C. and Ransohoff, J. F., Preferential induction of central or peripheral nervous system tumors in rats by nitrosourea derivatives, J Neuropathol Exp Neurol **33** 595-615, 1974.
4) Dittmann, L., Axelsen, N. H., Nørgaard-Pedersen, B. and Bock, E., Antigens in human glioblastomas and menigiomas : Search for tumour and onco-foetal anigens ; Estimation of S-100 and GFA protein, Brit J Cancer **35** : 135-141, 1977.
5) Druckley, H., Steinhoff, D., Preussmann, R. and Ivankovic, S., Erzeugung von Krebs durch eine einmalige Dosis von Methylnitroso-Harnstoff and verschiedenen Dialkylnitrosaminen in Ratten, Z Krebsforsch **66** 1-10, 1964.
6) Fitzgerald, J. E., Schardeim, J. L. and Kurtz, S. M., Spontaneous tumors of the nervous system in albino rats, J Natl Cancer Inst **52** : 265-267, 1974.
7) Goth, R. and Rajewsky, M. F., Molecular and cellular mechanisms associated with pulse carcinogenesis in the rat nervous system by ethylnitrosourea · Ethylation of nucleic acids and elimination rates of ethylated basies from the DNA of different tissues, Z Krebsforsch **82** : 37-64, 1974.
8) Knox, P., Carcinogenic nitrosoamides and cell cultures, Nature **256** : 671-673, 1976.
9) Lageman, A. and Dietz, W., Zur Feinstruktur fruehen Veraederungen an Rattenhirngewebe nach Injection von Methlylnitrosoharnstoff, Experimentia **28** : 1344-1345, 1972.
10) Lantos, P. L., The fine structure of perivenricular pleomorphic glioma induced transplacentally by N-ethyl-N-nitrosourea in BD-IX rats ; with a note on their origin, J Neurol Sci **17** : 443-460, 1972.
11) Lantos, P. L., Fine structural localization of acid phosphatase in neural tumors induced by N-ethyl-N-nitrosourea in rats, Acta Neuropathol (Berl.) **29** : 45-55, 1974.
12) Magee, P. N. and Barnes, J. M., The production of malignant primary hepatic tumors in the rat fed by dimethylnitrosamine, Brit J Cancer **10** : 114-122, 1956.
13) Napalkov, N. P. and Alexandrov, V. A., Neurotropic effect of 7, 12-dimethylbenzanthracene in transplacental carcinogenesis, J Natl Cancer Inst **52** : 1365-1366, 1974.
14) Rubinstein, L. J., Herman, M. M., Studies on the differentiation of human and experimental gliomas in organ culture systems, Recent Result Cancer Res **51** : 35-51, 1975.
15) Schreiber, D., Batka, H. and Lageman, A., Hormonelle Stoerungen und Geschwulstentwicklelung in Zentral Nerven System Experimentelle Untersuchungen an Ratten, Zbl Allg Pathol **117** : 180-184, 1973.
16) Stavrou, D., Zur Morphologie und Histochemie experimentelle induzierter Hirntumoren beim Kaninchen, Z Krebsforsch **73** : 98-109, 1969.
17) Stavrou, D., Haglid, K. G. and Weidenbach, W., The brain specific S-100 and 14-3-2 in experimental brain tumors of rat, Z Ges Exp Med **156** : 237-242, 1971.
18) Stavrou, D., Haglid, K. G., Zankl, H. and Zang, K. D., Immunhistochemischer Nachweis des hirnspecifischen S-100 Proteins bei experimentelle Hirntumoren, Z Krebsforsch **82** 75-82, 1974.
19) Swenberg, J. A., Koestner, A., Wechsler, W., Brunden, M. N. and Abe, H., Differential oncogenic effects of methynitrosourea, J Natl Cancer Inst **54** : 89-96, 1975.
20) Swenberg, J. A., Clendenon, N., Denlinger, R. and Gordon, W. A., Sequential development of ethynitrosourea-induced neurinomas : Morphology, Biochemistry and Transplantability, J Natl Cancer Inst **55** : 147-152, 1975.
21) Warzok, R., Schneider, J., Schreiber, D. and Jänisch, W., Experimental brain tumors in dogs. Experimentia **26** : 303-304, 1970.
22) Wechsler, W., Kleinhus, P. Matsumoto, S., Zuelch, K. J. et al., Pathology of experimental neurogenic tumors chemically induced during prenatal and postnatal life, Ann N Y Acad Sci **159** : 360-408, 1969.
23) Wechsler, W., Pfeiffer, S. E., Swenberg, J. A. and Koestner, A., S-100 protein in methyl- and ethylnitrosourea induced tumors of the rat nervous system, Acta Neuropathol (Berl) **24** : 287-303, 1973.

和文抄録

エチルニトロソウレアによる神経系腫瘍の誘発と，その被移植性

京都大学医学部脳神経外科学教室（主任：半田 肇教授）

織田祥史，半田 肇

Fibiger Laboratoriet, Danmark

Jørgen KIELER

胎生第Ⅲ期の Wistar Rat に ethylnitrosourea を経胎盤投与し，3ケ月以上生存した出生仔110匹中，96匹に神経系腫瘍ができた．

組織学的に41%が malignant neurinoma，25%が melignant oligadendroglioma であった．

同様の投与法で dimethylbenzanthracene を投与しても神経系腫瘍はできなかった．

発生した ENU-神経系腫瘍のうち7例が in viro で継代移植され，成ラットの皮下に維持された．第2代目から潜伏期，生存期間ともに，ほぼ一定となる傾向にあった．

たとえ組織学的に類似した腫瘍であっても，その成長速度に著明な差異をしめすものがあり，これらの差は，各種腫瘍間の抗原性の強弱に由来するものと推定された．